

SUPPLEMENTARY INFORMATION

Glucocorticoid agonists enhance retinal stem cell self-renewal and proliferation

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SUPPLEMENTARY FIGURES

Figure S1., Figure S2., Figure S3., Figure S4., Figure S5.

SUPPLEMENTARY TABLES

Table S1., Table S2.

SUPPLEMENTARY FIGURES

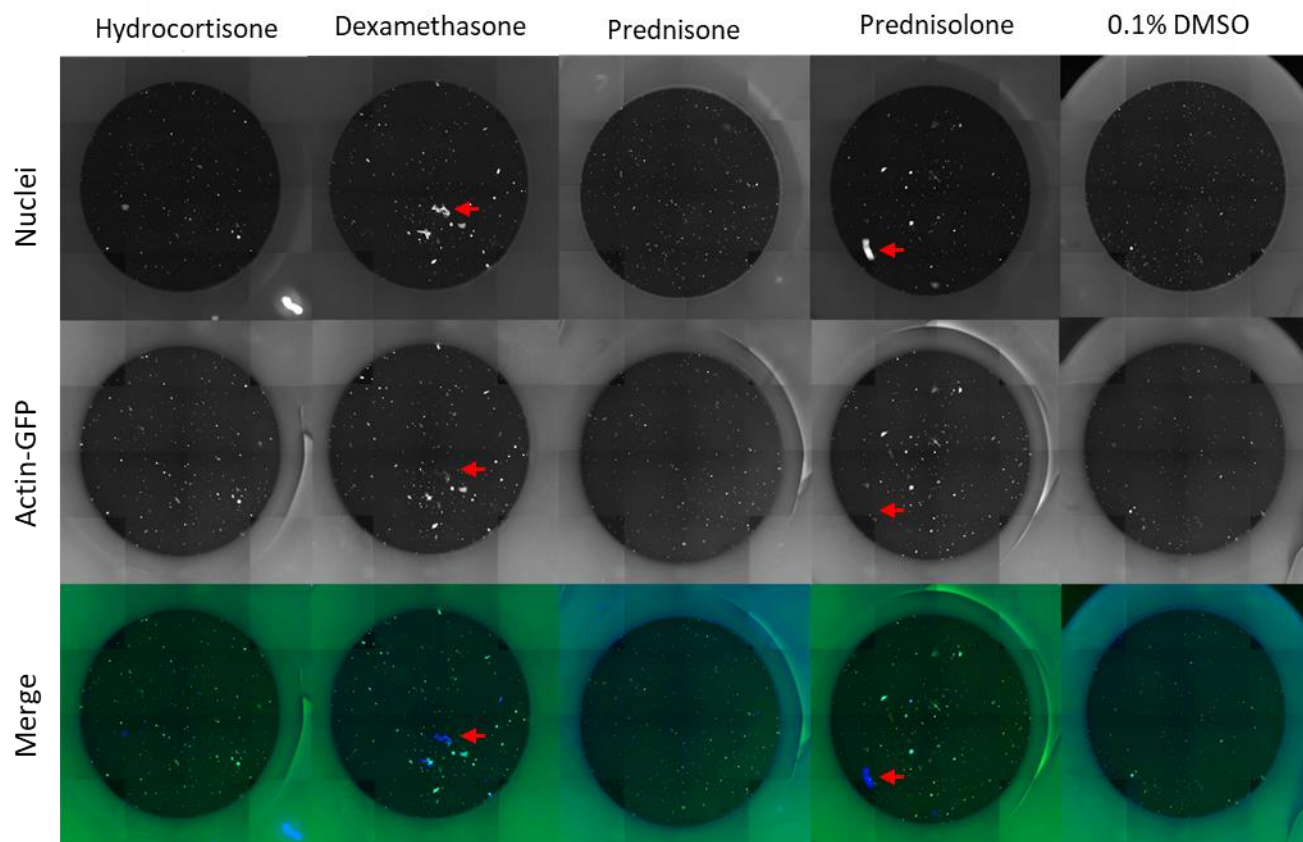


Figure S1. Visual confirmation that glucocorticoid agonists enhanced retinal stem and progenitor yield and was not due to artifacts

Images from the Celigo imaging cytometer showing 96-well plate wells at the end of a 7-day growth assay. Wells were treated with the indicated glucocorticoid agonist compounds. The nuclear channel, the GFP channel and merge demonstrate the ability to differentiate Hoechst and actin-GFP double-positive objects from debris and other artifacts that appear only in the nuclear channel. Visually, it is apparent dexamethasone and prednisolone have greater signal than the other conditions. Red arrows indicate artifacts that fluoresce in the blue nuclear channel that do not fluoresce in the GFP channel. 4x magnification.

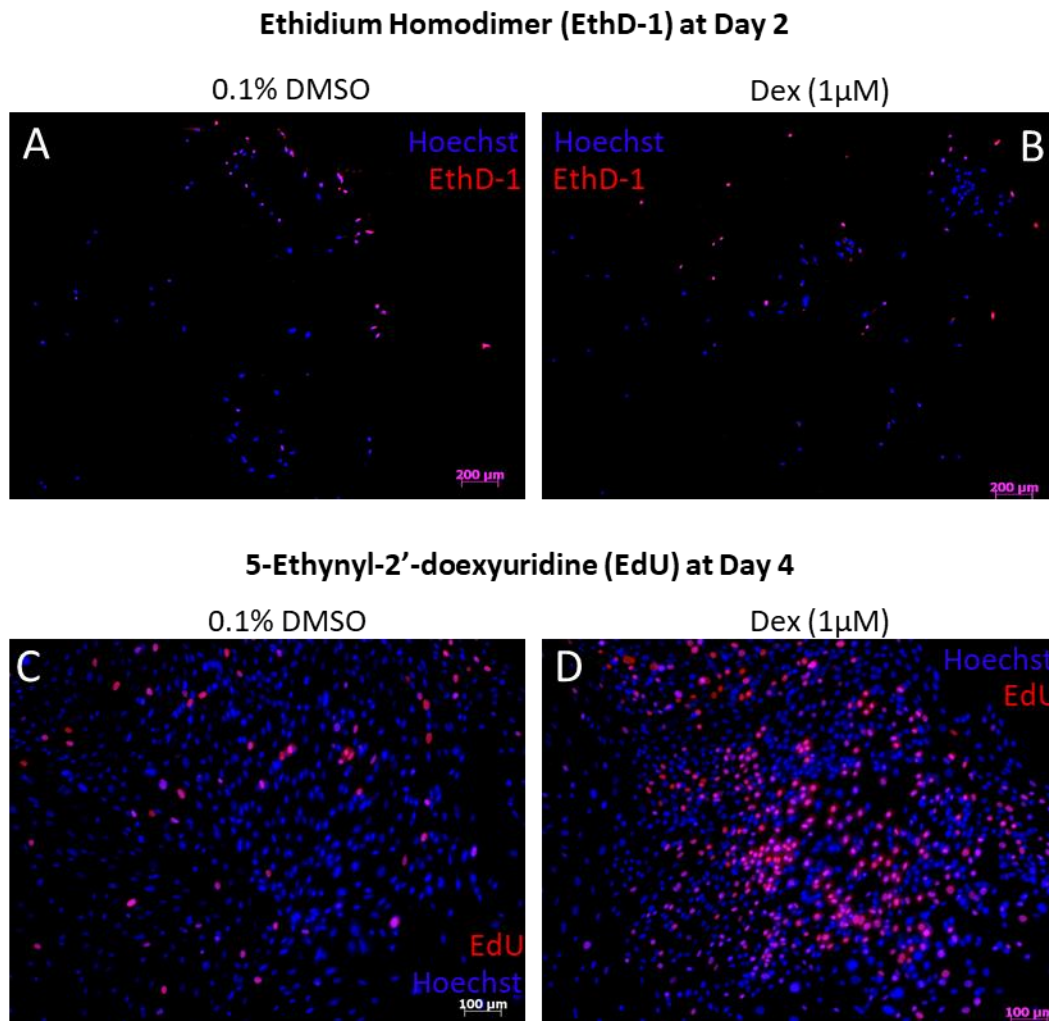


Figure S2. Representative staining of cell death marker ethidium homodimer (EthD-1) and thymidine analog EdU.

(A-B) EthD-1 labeling at Day 2 in cells treated with **(A)** 0.1% DMSO, or **(B)** 1 μ M Dexamethasone.

(C-D) EdU labeling at Day 4 in cells treated with **(A)** 0.1% DMSO, or **(B)** 1 μ M Dexamethasone.

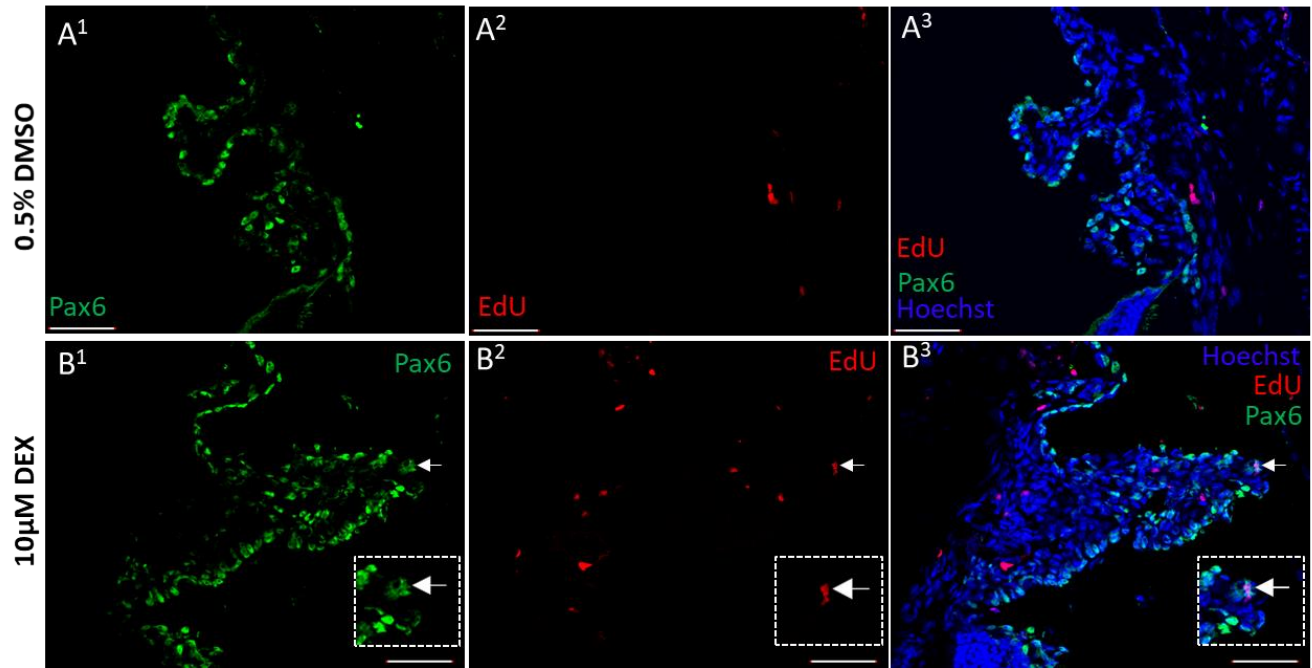


Figure S3. Intravitreal dexamethasone injection induces ciliary epithelium proliferation

(A-B) Representative images of Pax6 IHC and EdU labeling in the ciliary body of mouse eyes exposed to **(A)** 0.5% DMSO vehicle, or **(B)** 1µM Dexamethasone. Nuclei are labeled via Hoechst staining. White arrows indicate Pax6 + EdU co-labeled cells. Dashed line indicates inset. 10 µm-thick sections.

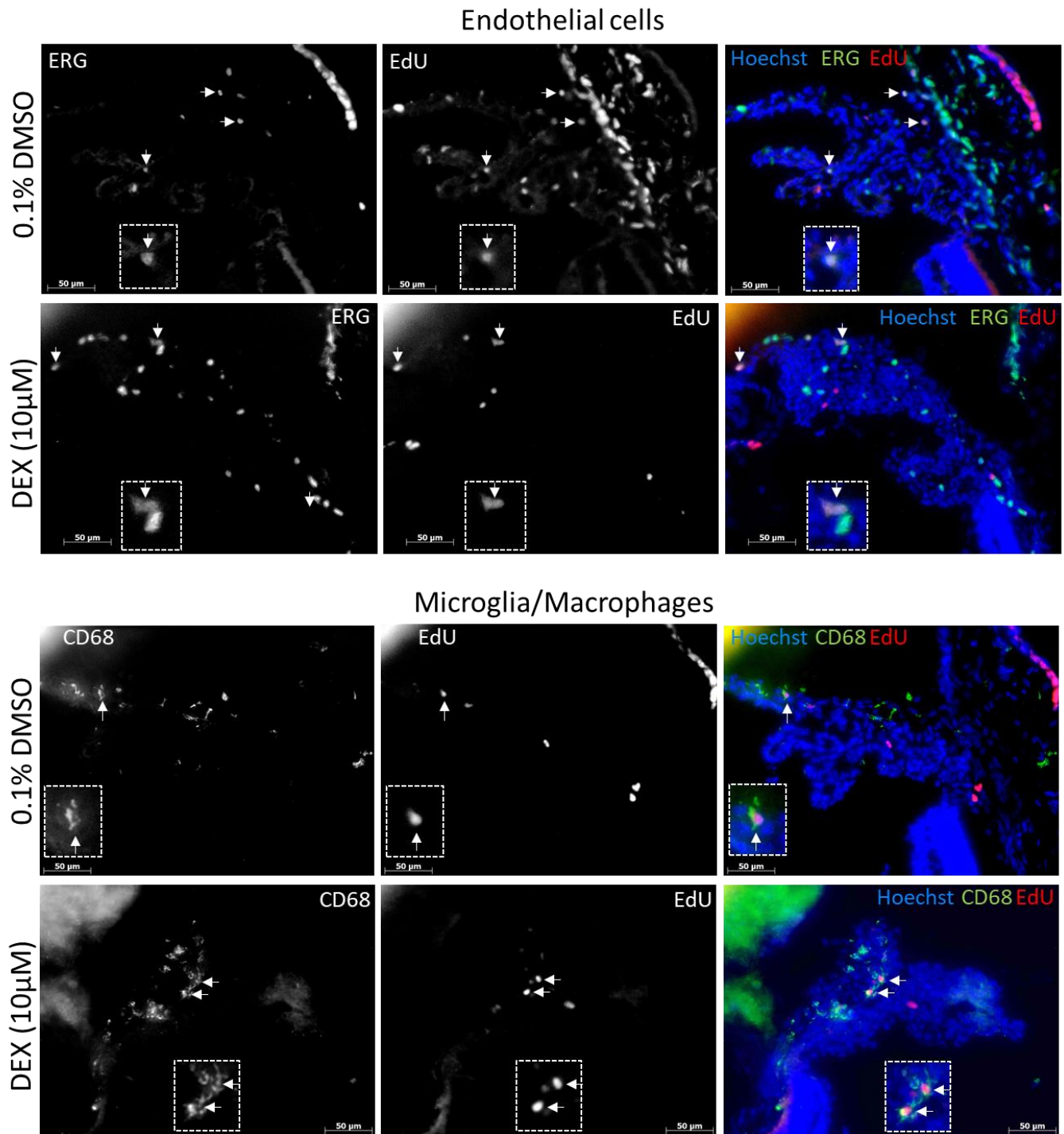


Figure S4. EdU-positive cells co-label with endothelial and microglia/macrophage markers

(A-B) Representative images of ERG IHC and EdU labeling in the ciliary body of mouse eyes exposed to **(A)** 0.5% DMSO vehicle, or **(B)** 1 μ M Dexamethasone.

(C-D) Representative images of CD68 IHC and EdU labeling in the ciliary body of mouse eyes exposed to **(C)** 0.5% DMSO vehicle, or **(D)** 1 μ M Dexamethasone. Nuclei are labeled via Hoechst staining. White arrows indicate co-labeled cells. 10 μ m-thick sections.

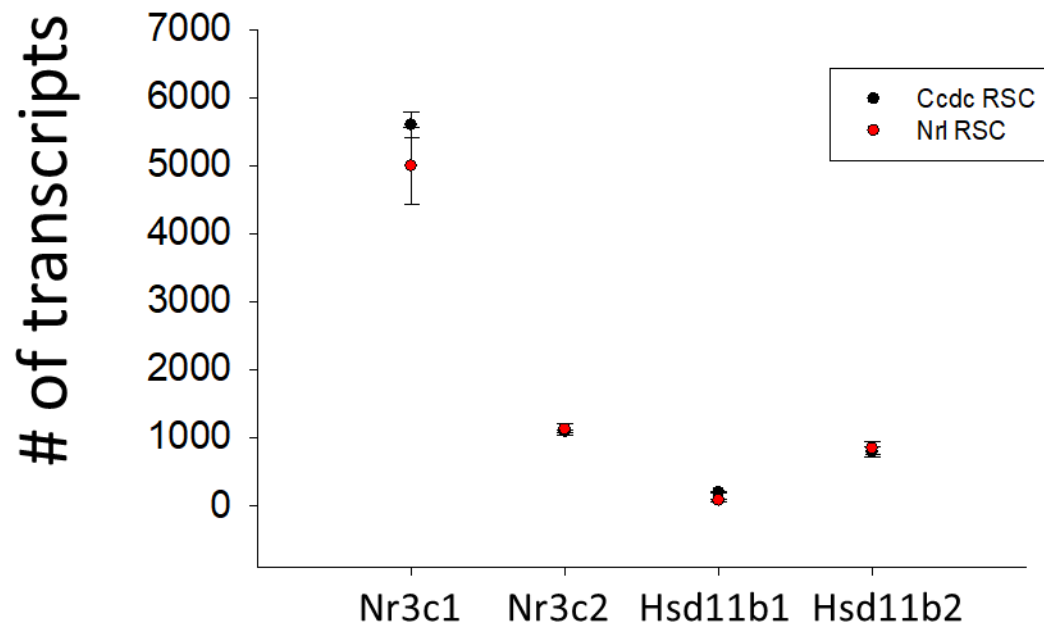


Figure S5. Glucocorticoid receptor, Mineralocorticoid receptor, and 11- β -HSD1 & 2 RNA expression in RSC spheres

Transcriptomic data showing the expression of the glucocorticoid receptor (Nr3c1), mineralocorticoid receptor (Nr3c2) and the two 11- β -HSD isozymes in RSC spheres, supporting the finding of retinal precursor sensitivity to GR agonists. This graph was created from RNAseq data collected in Khalili et al. 2018. Two different mouse strains were used to generate RSC spheres that were lysed and high-quality total RNA (RIN: 9–10) was subjected to directional RNA-sequencing library construction from three independent biological replicates per mouse strain. Sequencing was performed using GAllx (Illumina, Inc., San Diego, CA; www.illumina.com).

SUPPLEMENTARY TABLES

Table S1. Compounds that met hit criteria in at least one of two screens

| Compound | Mechanism | Mechanism Hit Frequency | | Screen 1 Hit | | | Screen 2 Hit | | |
|----------------------|--|-------------------------|--------|--------------|------|-------|--------------|------|-------|
| | | Freq. | P<0.05 | Nuclei | GFP | Ratio | Nuclei | GFP | Ratio |
| Prednisolone | Glucocorticoid receptors alpha and beta agonist | 2/4 | √ | 145% | 160% | 1.10 | 158% | 162% | 1.02 |
| Dexamethasone | Glucocorticoid receptors alpha and beta agonist | 2/4 | √ | 142% | 150% | 1.05 | | | |
| MK-0431 | Dipeptidyl peptidase IV (CD26; DPP-IV) inhibitor | 1/1 | √ | 150% | 137% | 1.09 | | | |
| MDV-3100 | Androgen receptor antagonist | 1/5 | | 139% | 189% | 1.36 | * | * | |
| Abbvie Mcl-1 Cpd 30b | Mcl-1 inhibitor | 1/5 | | 140% | 148% | 1.06 | | | |
| Sodium Butyrate | HDAC inhibitor | 1/47 | | 134% | 208% | 1.55 | * | * | |
| Odanacatib | Cathepsin K inhibitor | 1/1 | √ | 136% | 240% | 1.76 | | | |
| INCB024360 | Indoleamine 2,3-dioxygenase inhibitor | 1/1 | √ | | | | 143 | 139% | 0.97 |
| Thiazovivin | Rho/ROCK inhibitor | 1/3 | | | | | 140% | 132% | 0.94 |
| Lopinavir | HIV protease inhibitor | 1/1 | √ | | | | 129% | 254% | 1.97 |
| Guanabenz Acetate | A2-adrenergic receptor agonist | 1/1 | √ | | | | 129% | 142% | 1.10 |
| Irrestatin 9389 | IRE1 inhibitor | 1/3 | | | | | 137% | 143% | 1.04 |

* = seeding error: compound not in second screen

List of hit compounds and their known signaling pathways/mechanistic targets. Mechanism hit frequency indicates the number of hit compounds with the indicated mechanistic target relative to the total number of compounds with that target in the library. P-values are indicated for any target pathway with a significant enrichment within the library, as determined by the hypergeometric test. Percentages represent the number of nuclei or total actin-GFP area relative to 0.1% DMSO control (set as 100%). Percent of control is only indicated if a compound was found to be greater than three standard deviations above the control mean for both nuclei number and GFP area within a single screen. Ratio indicates the GFP area relative to the number of nuclei. A large GFP/Nuclei ratio indicates a disproportionate increase in cell area vs cell number and may signify compounds that caused cell hypertrophy.

Table S2. Screening quality metrics

| | Screen 1 | | Screen 2 | |
|---------------------------------|-------------------|---------------------|-------------------|---------------------|
| | Nuclei | GFP | Nuclei | GFP |
| SSMD | -4.30 (Strong) | -2.83 (Moderate) | -3.75 (Strong) | -2.55 (Moderate) |
| Coefficient of Variation | 13.92 | 11.51 | 7.97 | 10.21 |
| Signal:Noise | 8.54 | 10.9 | 18.08 | 4.16 |

Statistical evaluation of the variability and sensitivity of the MTS assay. Controls were determined to meet a threshold of statistical confidence to reliably determine hit compounds by assessing the variability within the 1x Control (coefficient of variation) and comparing the 1x Control with the pseudo-positive 2x Control (signal:noise ratio; strictly standardized mean difference (SSMD)). If SSMD is ≤ -2 and > -3 it is considered a moderate control. If SSMD is ≤ -3 and > -5 it is considered a strong control.